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# Chemokines in onchocerciasis patients after a single dose of ivermectin

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## Summary

**Ivermectin treatment will effectively diminish microfilariae (Mf) of *Onchocerca volvulus* in the skin of patients, but therapy is associated with adverse host inflammatory responses. To investigate the association of proinflammatory chemokines with the intensity of infection and clinical adverse reactions, chemokine serum levels were measured in patients following ivermectin treatment (100 μg/kg, 150 μg/kg or 200 μg/kg) or placebo. The density of *O. volvulus* Mf per mg skin decreased by 85%, 97%, 97% and 90% at day 3, at month 3, month 6 and at 1 year post-ivermectin. The cutaneous T cell-attracting chemokine (CTACK/CCL27) was found highly elevated in onchocerciasis patients compared to infection-free European controls ($P = 0{\cdot}0004$) and it did not change following ivermectin or placebo to 1 year post-therapy. The chemokine RANTES/CCL5 (regulated on activated and normally T cell-expressed) was similarly high in onchocerciasis patients and infection-free European controls; the RANTES/CCL5 levels did not change following treatment until 6 months post-therapy but were slightly elevated at 1 year post-therapy ($P < 0{\cdot}02$). In contrast, the Th2-type chemoattractants, thymus and activation regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22), were activated at 3 days post-ivermectin ($P < 0{\cdot}0001$) to return to pretreatment or lower levels thereafter. The Th1-type chemoattractants, macrophage inflammatory protein (MIP)-1α/CCL3 and MIP-1β/CCL4 were low before ivermectin treatment, but following clearance of microfilariae of *O. volvulus* their levels increased from 6 months post-therapy onwards (for both at 12 months post-therapy, $P < 0{\cdot}0001$). The adverse reaction scores (RS) in treated patients increased significantly on day 3 ($P < 0{\cdot}02$) while it remained unchanged in those who received placebo ($P = 0{\cdot}22$); RS interacted with the microfilarial density ($P = 0{\cdot}01$), but not with the dose of ivermectin or with the serum levels of MIP-1α/CCL3, MIP-1β/CCL4, TARC/CCL17, MDC/CCL22 and CTACK/CCL27. Our observations suggest that following ivermectin, macrophages as well as memory Th2-type lymphocytes and B cells, attracted and activated by MDC/CCL22, TARC/CCL17 and CTACK/CCL27, may contribute to dermal immune responses and *O. volvulus* Mf killing and clearance. The transient changes of TARC/CCL17 and MDC/CCL22 were not associated with clinical adverse responses, and the later rise of MIP-1α/CCL3 and MIP-1β/CCL4 showed a reactivation of Type 1 immune responses associated with persistent low levels of *O. volvulus* microfilariae and an expiring *O. volvulus* infection.**

**Keywords:** chemokines, ivermectin treatment, *Onchocerca volvulus* infection

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## Introduction

In onchocerciasis, elimination of skin-dwellling microfilariae (Mf) is achieved efficiently by ivermectin treatment, but some patients develop skin rash, oedema, lymph node pain or swelling and fever [1–3]. By its mode of action ivermectin will inhibit the motility of Mf [4], and following their disappearance from the skin, large numbers of Mf will be found in regional lymph nodes where they become surrounded by eosinophils and macrophages [5–7]. Monocytes, lymphocytes, eosinophils and basophils are recruited and activated by chemokines [8]; these chemotactic cytokines are not only responsible for the trafficking of leucocytes to specific locations within the body. Chemokine-triggered cellular degranulation may result in inflammatory tissue destruction, chronic inflammatory disorders, airway inflammation and allergic processes [8]. In rheumatoid arthritis, asthma, atopy and myocardial infarction, chemokines are important regulators of inflammation [9], but comparatively little is known about their importance and function during parasite infections and to what extent chemokines contribute to infection-associated pathology or immune protection against parasites. In ivermectin-treated onchocerciasis patients, the onset of systemic adverse reactions was found associated with elevated plasma levels of interleukin (IL)-5, eosinophil-derived neurotoxin (EDN), eotaxin (CCL11) and RANTES (regulated on activated and normally T cell-expressed) (CCL5) and these chemotactic cytokines were suggested to participate in eosinophil recruitment and cellular killing of microfilariae of *Onchocerca volvulus* [10,11]. However, both beneficial as well as unwanted adverse responses may develop following anti-parasite treatment [2,12]. The balance between regulatory and inflammatory immune mediators, notably cytokines and chemokines, may determine the degree of adverse side effects and disease expression [8–11]. With chronic helminth parasite infections, eosinophilia, elevated IgE and $IgG_4$, dominant Th2-cytokine responses – notably IL-4 and IL-5 – will develop and such reactivity is also recognized as a characteristic trait of allergic diseases. Anti-helminth treatment will decrease dominant Th2-type cytokine responses [13–15] and similarly, in allergy patients, specific immunotherapy will generate Th1-type cytokines, especially interferon (IFN)-γ and $IgG_4$ responses, resulting in a mixed Th Type 1 and Type 2 immune response profile which prevents an allergic sequel [16,17]. Because immune responses beneficial against parasites may cause immune pathology to the host, an appropriate anti-parasite immunity requires finely balanced and also, to some extent, compromised immune responses to avoid host damage.

As observed in the present study, the Th2-type chemokines MDC/CCL22 and TARC/CCL17 increased temporarily and shortly after ivermectin treatment – these may have contributed to the rapid elimination of Mf of *O. volvulus* from the skin, while the later rise of the Th1-type chemokines macrophage inflammatory protein (MIP)1-α and MIP1-β appeared to be associated with persistent low levels of Mf and an expiring *O. volvulus* infection.

## Materials and methods

### Patients

All patients from this study originated from villages in the central region of Togo, where *O. volvulus* was hyper- to mesoendemic. Patients were apparently healthy males and non-pregnant women with a body weight over 30 kg, without history of multiple allergies or drug intolerance, with normal laboratory parameters except for the clinical features of onchocerciasis, with skin biopsies positive for microfilaria of *O. volvulus* [2] and with palpable nodules (onchocercomata). Criteria for exclusion were allergies or drug intolerance, concomitant infection with *Loa loa* or *Wuchereria bancrofti,* haematocrit below 30%, renal or hepatic disease, convulsions or other central nervous system (CNS) disease, clinical signs suggestive of meningitis, pregnant females or nursing mothers and patients who had received microfilaricidal drugs during the preceding year. Patients were enrolled in the phase III double-blind placebo-controlled dose-finding study of ivermectin for treatment of onchocerciasis. The protocol of the study was reviewed and approved by the Ethics Commission of the Medical Board at University of Tübingen, the Advisory Council of the Ministry of Health in Togo and the Committee on Research Involving Human subjects of the World Health Organization.

### Patient groups and examination during and after chemotherapy

Four treatment groups were formed, as indicated in Table 1. In patients, the densities of microfilaria of *O. volvulus* (Mf/mg) in the skin were determined at the right and left iliac crest and the right and left calf by means of corneascleral punch (Holth- or Walser-type) before ivermectin treatment and at day 3, at month 3, month 6 and at 1 year post-treatment. Skin biopsies were immediately weighed and then immersed in 0·1 ml physiological saline in a well in a flat-bottomed microtitre plate, and stored at room temperature in a high-humidity atmosphere. Microfilariae were counted after overnight incubation. Patients were treated with ivermectin at 100 μg/kg, 150 μg/kg and 200 μg/kg or received placebo and at the above dates patients were examined physically and intensity of signs and symptoms following ivermectin or placebo were scored, as described in detail previously by Greene *et al.* [1]. A grading scale for clinical reaction score (from 0 to 3) was applied for skin rash type (none, oedema, popular, pustular), extent of rash (none, less than one-third, more than one-third, whole body) and pruritis (none, mild, marked, marked with frank excoriation), cervical, axillar, inguinal/femoral and other lymph node

**Table 1.** Characteristics of patient groups and treatment.

| | Patient groups | | | |
|---|---|---|---|---|
| | Ivermectin | | | |
| | 100 μg/kg ($n = 13$) | 150 μg/kg ($n = 8$) | 200 μg/kg ($n = 24$) | Placebo ($n = 37$) |
| Sex (male/female) | 12/1 | 8/0 | 21/3 | 32/5 |
| Age (median) (range) | 30 (12–46) | 20 (15–37) | 38 (12–52) | 33 (15–60) |
| Mf/mg† day 0 (mean) (range) | 68·9 (12·1–184·7) | 62·9 (6·6–148·9) | 79·2 (0·4–345·6) | 62·9 (0–302·2) |
| Mf/mg day 3 (range) | 17·1** (1·7–62·5) | 10·7** (0·2–60) | 8·4*** (0·4–49·9) | 62·7 (0·5–177·3) |
| Reaction score day 0 (mean) (range) | 2·6 (1–6) | 1·5 (1–5) | 2·3 (0–7) | 2·2 (0–7) |
| Reaction score day 3 (range) | 3·5 (1–8) | 3·5** (1–7) | 3·4* (1–7) | 1·9 (0–6) |

†Mf/mg = microfilariae of *Onchocerca volvulus* per mg skin biopsy. *Onchocerca volvulus* microfilariae densities (Mf/mg skin biopsy) and clinical reaction scores were determined in onchocerciasis patients before and at day 3 post-ivermectin treatment or placebo. Patients were treated with 100 μg/kg, 150 μg/kg, 200 μg/kg ivermectin or placebo. Patients were examined, treated and followed-up as described in Materials and methods. Significant differences before and post-treatment are indicated as $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ and $^{***}P < 0{\cdot}0001$.

enlargement (none, mild, moderate, severe) and lymph node tenderness (none, mild, marked, pain), arthralgia or synovitis (none, pain, pain with motion limitation, effusion/swelling) and fever (<38°C, 38–38·4°C, 38·5–39°C, >39°C) and from this cumulative reaction scores – the total of the seven values – were calculated for each patient and for each day of examination. Following each physical examination 5–10 ml of venous blood was collected for clinical laboratory parameter determination and serum samples were aliquoted and stored at below −24°C and for long-term storage at −80°C until further use.

### Quantification of chemokines

Levels of MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, TARC/CCL17, MDC/CCL22 and CTACK/CCL27 were measured by specific sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Wiesbaden, Germany), as recommended by the manufacturer. The detection limits for MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, TARC/CCLL17 and MDC/CCL22 were 10 pg/ml and for CTACK/CCL27 was 50 pg/ml.

### Data analyses

For data analyses the statistical package JMP 5·0.1·2 was used. The data given as box-plots show the median with the 25% and 75% quartiles and 1·5× of the interquartile range. Clinical data of all patients were gathered in a double-blind fashion, those of the ivermectin and placebo patients during the first 12 months after therapy. Comparison of more than two means was calculated using one-way analysis of variance (ANOVA) for independent groups and ANOVA for repeated measurements for paired data. Chemokine production post-treatment was analysed by multiple regression analysis. The analysis involved the predictors chemokine concentrations, treatment group, time post-treatment, reaction score, microfilarial densities and their corresponding interactions of degree 2.

## Results

In ivermectin-treated patients ($n = 45$) the density of *O. volvulus* microfilariae (Mf) per mg skin (before: mean 73·4 Mf/mg) decreased by 85% (11·2 Mf/mg), 97% (2·1 Mf/mg), 97% (2·4 Mf/mg) and 90% (7·7 Mf/mg) on day 3, month 3, month 6 and 1 year post-treatment, respectively, while in placebo patients ($n = 37$) microfilarial densities on day 0 (mean 62·9 Mf/mg; range 0–302·2 Mf/mg) did not diminish by 1 year later (mean 40·1 Mf/mg; range 0·7–145·6 Mf/mg). In onchocerciasis patients' serum levels of MIP-1α/CCL3, MIP-1β/CCL4, TARC/CCL17, MDC/CCL22, CTACK/CCL27 and RANTES/CCL5 were determined following ivermectin therapy (100 μg/kg, 150 μg/kg or 200 μg/kg) or placebo. Chemokine concentrations were measured before (d0), 3 days (d3), 3 months (m3), 6 months (m6) and 1 year (y1) post-therapy and were compared to placebo-treated onchocerciasis patients and infection-free European over-sea travellers ($n = 25$). The levels and changes of chemokines following ivermectin are shown in Fig. 1.

For MIP-1α/CCL3, before and at 3 days post-intervention, the concentration of MIP-1α/CCL3 remained low in ivermectin-treated (median 73 pg/ml) and placebo-treated (median 82 pg/ml) onchocerciasis patients as well

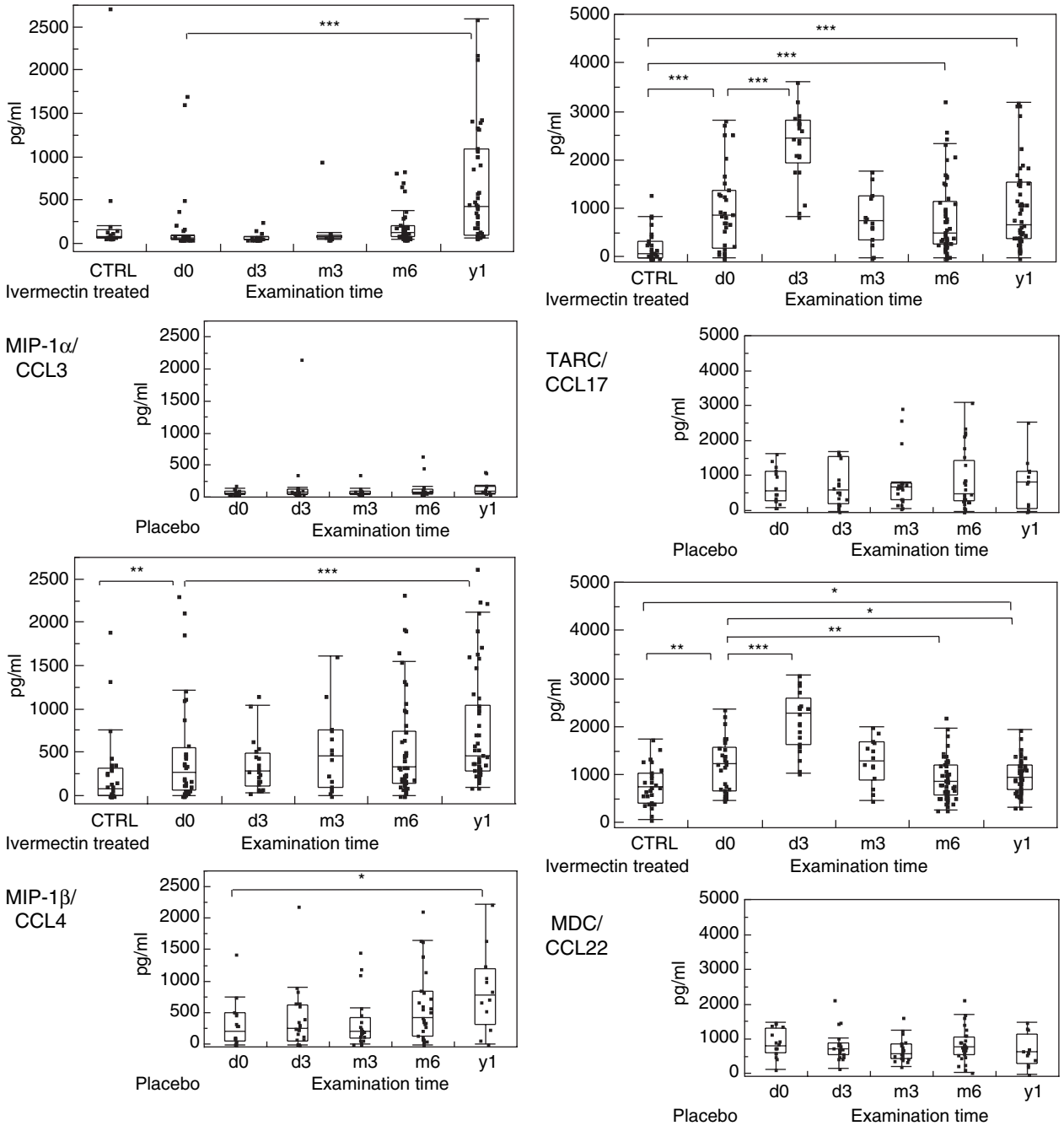


**Fig. 1.** Chemokine levels in ivermectin-treated onchocerciasis patients and controls. Concentration of MIP-1α/CCL3, MIP-1β/CCL4, TARC/CCL17, MDC/CCL22, CTACK/CCL27 and RANTES/CCL5 in sera from onchocerciasis patients treated with ivemectin, from patients given placebo and from infection-free European controls (CTRL). Treatment groups given 100 μg/kg, 150 μg/kg and 200 μg/kg ivermectin were pooled. Chemokine concentrations were determined (examination time) at before treatment (d0), 3 days (d3), 3 months (m3), 6 months (m6) and 1 year (y1) after therapy. Boxplots show the median with the 25% and 75% quartiles and 1·5× of the interquartile range. Significant differences are indicated as $^{*}P < 0{\cdot}05$, $^{**}P \leq 0{\cdot}01$, $^{***}P \leq 0{\cdot}0001$.

as in infection-free controls (median 83 pg/ml). For ivermectin-treated patients, MIP-1α/CCL3 was between 51 and 1714 pg/ml and for placebo-treated patients between 53 and 185 pg/ml, and the levels did not change from before and at month 3 post-ivermectin or placebo in either group. At 6 months post-ivermectin, serum MIP-1α/CCL3 content increased slightly (median 132 pg/ml), while a significant increase ($P < 0{\cdot}0001$) was observed (median 424 pg/ml, max 4315 pg/ml) to 1 year post-therapy. In the placebo group MIP-1α/CCL3 serum levels did not change at day 0, day 3,

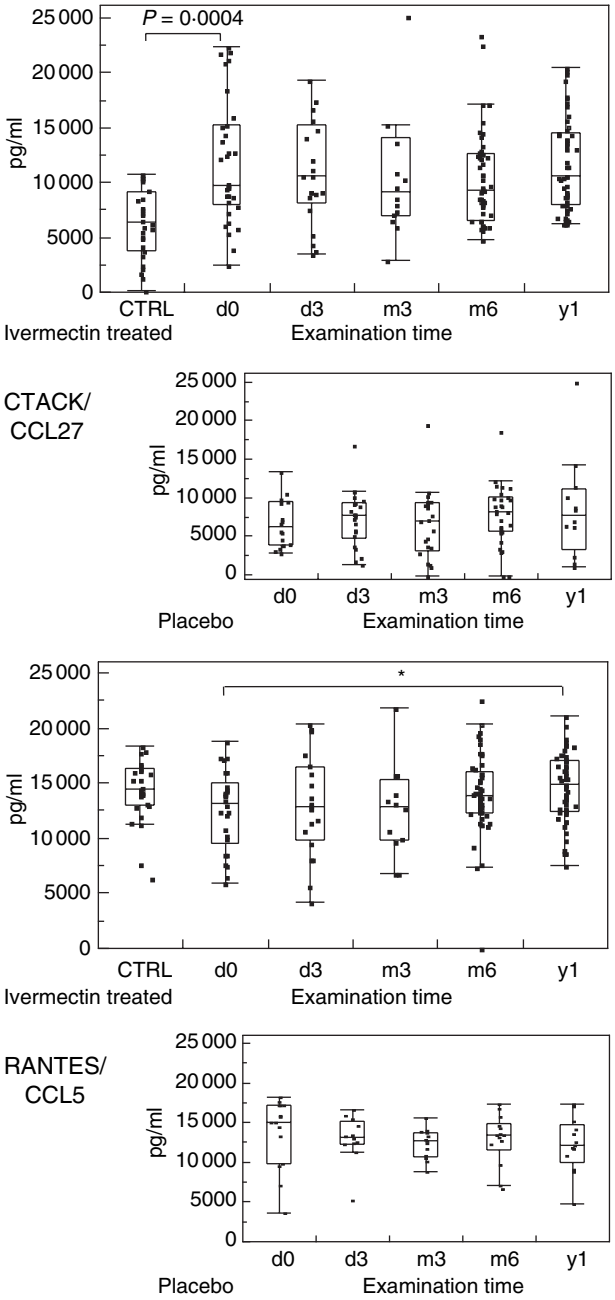


**Fig. 1.** *Continued*

at month 3, month 6 and at 1 year post-therapy (median 98 pg/ml).

For MIP-1β/CCL4, the serum concentrations (median 86 pg/ml) remained significantly lower ($P = 0{\cdot}01$) in infection-free controls than in untreated patients. The pretreatment MIP-1β/CCL4 concentrations were higher (median 269 pg/ml, maximum 2321 pg/ml) than those for MIP-1α/CCL3, but a similar kinetics was found following therapy. In ivermectin-treated patients, MIP-1β/CCL4 remained unchanged at day 3 post-therapy; at 6 months post-therapy it increased to a median 336 pg/ml and reached 460 pg/ml at 1 year post-therapy ($P = 0{\cdot}0001$), which was similar to MIP-1α/CCL3. In the placebo group MIP-1β/CCL4 serum levels did not change until month 6 post-therapy but were slightly elevated at 1 year post-therapy.

Sera of untreated onchocerciasis patients contained significantly higher TARC/CCL17 (median 868 pg/ml, maximum 2835 pg/ml) than those from infection-free European controls (median 88 pg/ml, maximum 1304 pg/ml) ($P < 0{\cdot}0001$), which was also observed for MDC/CCL22. Similarly to MDC/CCL22, concentrations of TARC/CCL17 increased briskly (median 2475 pg/ml, maximum 3628 pg/ml) at day 3 post-therapy in patients ($P < 0{\cdot}0001$) and at 6 and 12 months reached post-therapy levels as observed before treatment (median <700 pg/ml), but still higher than in controls ($P < 0{\cdot}0001$). In the placebo group, day 0 and day 3 sera contained similar amounts of TARC/CCL17 (median 1174 pg/ml, maximum 1648 pg/ml) ($P = 0{\cdot}86$) and levels remained unchanged at 1 year post-therapy.

Before intervention, serum MDC/CCL22 was lower in controls (median 764 pg/ml, maximum 1754 pg/ml) ($P = 0{\cdot}01$) than in patients (median 1233 pg/ml, maximum 2382 pg/ml) and a different kinetic to MIP-1α/CCL3 and MIP-1β/CCL4 was observed for MDC/CCL22 following ivermectin. At 3 days post-therapy, MDC/CCL22 increased to 2291 pg/ml (median) (maximum 3084 pg/ml) ($P < 0{\cdot}0001$) and declined thereafter at 6 and 12 months post-therapy to concentrations lower than before ivermectin donation ($P = 0{\cdot}01$, $P = 0{\cdot}04$, respectively). In the placebo group, MDC/CCL22 levels remained unchanged from day 0 (median 766 pg/ml) to 1 year post-intervention (median 645 pg/ml).

The absolute amounts of CTACK/CCL27 in patients' sera were several magnitudes higher than those found for MIP-1α/CCL3, MIP-1β/CCL4, MDC/CCL22 and TARC/CCL17. In infection-free European controls, the maximum CTACK/CCL27 reached 10850 pg/ml while it was 22492 pg/ml in patients (median 9852 pg/ml) ($P = 0{\cdot}0004$). Following ivermectin, CTACK/CCL27 did not change significantly, with median values being 10732 pg/ml on day 3, then 9425 pg/ml at month 6 and 10700 pg/ml at 1 year post-treatment. In patients receiving placebo, mean CTACK/CCL27 concentrations in sera did not change ($P = 0{\cdot}94$) from day 0 (median 7365 pg/ml) to day 3 and at 1 year post-intervention (8683 pg/ml).

The chemokine RANTES/CCL5 (regulated on activated and normally T cell-expressed) was similarly high in onchocerciasis patients and infection-free European controls; the RANTES/CCL5 levels did not change following treatment until 6 months post-therapy but were slightly elevated at 1 year post-therapy ($P < 0{\cdot}02$). No changes of RANTES/CCL5 serum levels were observed in the placebo group patients.

The concentrations of MIP-1α/CCL3 and MIP-1β/CCL4 in sera did not correlate ($r^2 < 0{\cdot}1$) with any of the other chemokines studied but a positive correlation was observed

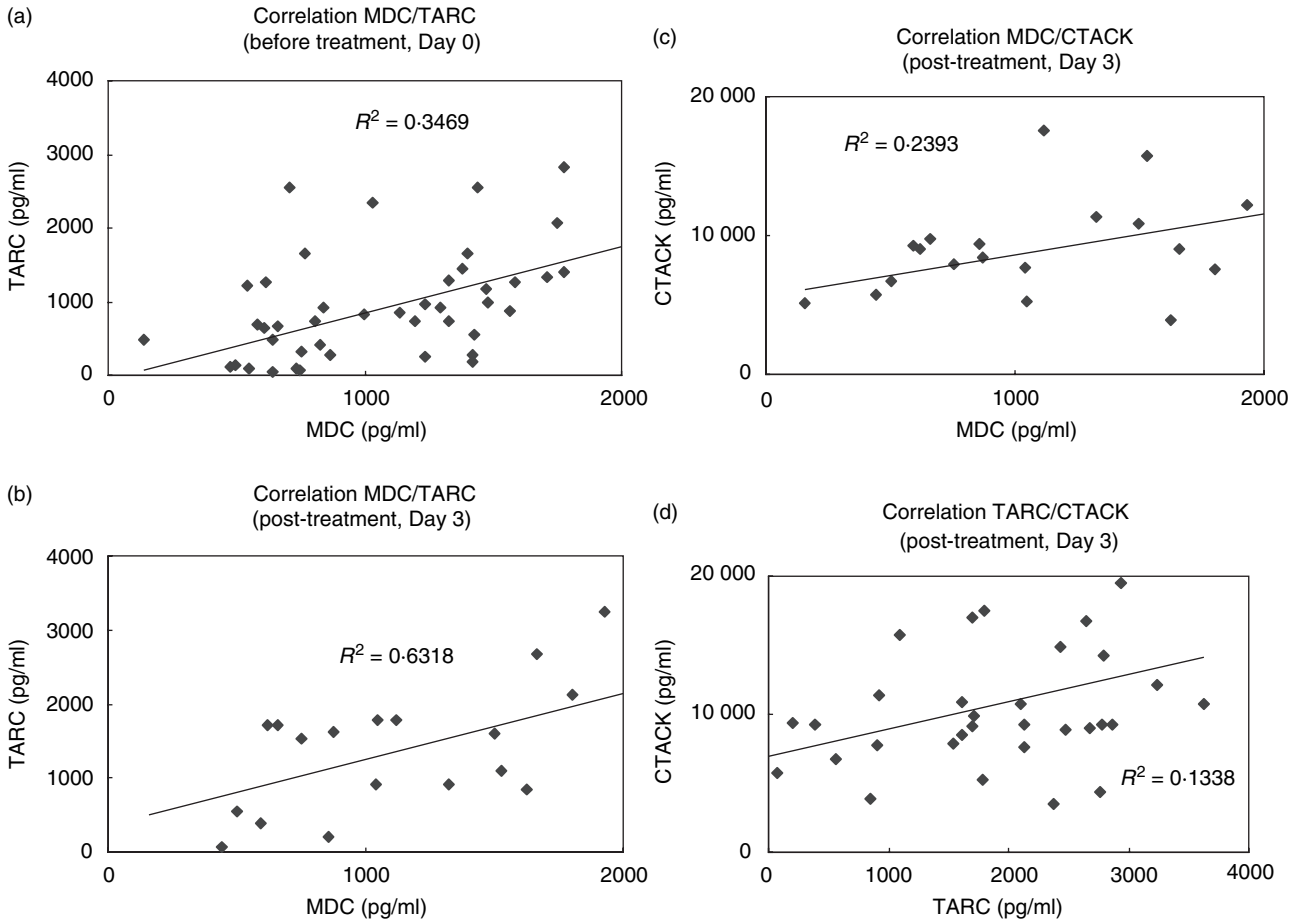


**Fig. 2.** Concentration and correlation MDC/CCL22 and TARC/CCL17 in onchocerciasis patients before (d0) (a) and 3 days (d3) (b) post-ivermectin treatment. Concentration and correlation of MDC/CCL22 and CTACK/CCL276 (c) as well as TARC/CCL17 and CTACK27 (d) 3 days (d3) post-ivermectin treatment. Serum chemokine concentrations were determined by enzyme-linked immunosorbent assay (ELISA) as described in Materials and methods.

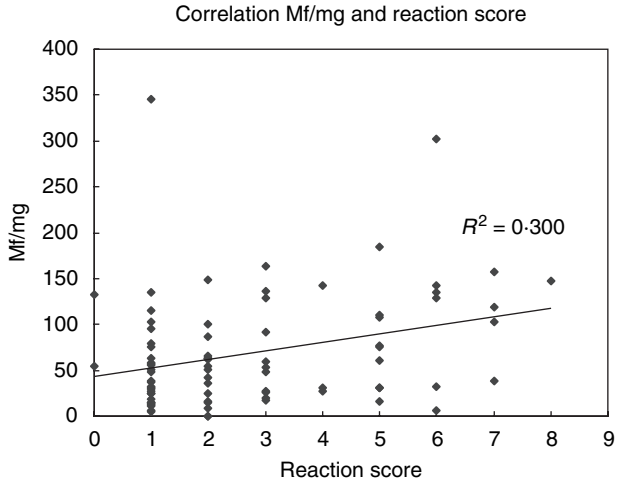


**Fig. 3.** Correlation of the density of microfilaria of *Onchocerca volvulus* (Mf/mg skin biopsy) before treament and the clinical adverse reaction scores (day 3 post-treatment) in onchocerciasis patients ($n = 82$) following ivermectin treatment (100 μg/kg, 150 μg/kg, 200 μg/kg) or placebo.

for MDC/CCL22 and TARC/CCL17, which was seen both before as well as 3 days post-treatment (Fig. 2). With increasing MDC/CCL22, serum levels of TARC/CCL17 were positively correlated at day 0 before treatment ($r^2 = 0{\cdot}347$) as well as 3 days post-ivermectin ($r^2 = 0{\cdot}632$). For MDC/CCL22 and CTACK/CCL27, only on day 3 did post-treatment elevated serum MDC/CCL22 correlate with CTACK/CCL27 ($r^2 = 0{\cdot}239$) and similarly, post-treatment TARC/CCL17 levels were positively correlated ($r^2 = 0{\cdot}134$) with CTACK/CCL27 (Fig. 2).

The adverse reaction scores in patients increased significantly on day 3 ($P < 0{\cdot}02$) following ivermectin while it remained unchanged in those who received placebo ($P = 0{\cdot}22$) (Table 1). Using multiple regression analysis, the adverse reaction scores were found to interact with the microfilarial density ($P = 0{\cdot}01$) (Fig. 3) but not with the dose of ivermectin (100 μg/kg, 150 μg/kg or 200 μg/kg). Also, adverse reaction scores did not interact with the serum levels of MIP-1α/CCL3, MIP-1β/CCL4, TARC/CCL17, MDC/CCL22 and CTACK/CCL27, and regression analysis of

chemokine levels following treatment disclosed that none interacted with microfilarial densities (Mf/mg). CTACK/CCL27 and MDC/CCL22 concentrations interacted with age ($P = 0{\cdot}03$, $P < 0{\cdot}001$, respectively), and only MDC/CCL22 levels showed a significant correlation with the dose of ivermectin ($P < 0{\cdot}001$).

## Discussion

Following treatment of onchocerciasis patients with a single dose of ivermectin, microfilariae (Mf) of *O. volvulus* will largely disappear from the skin within 2–3 days, but the effector mechanisms and factors which contribute to this Mf clearance remain only incompletely understood. While ivermectin will partially paralyse Mf [4], the sequestration of effector cells, notably macrophages and eosinophils, into dermal tissues and lymph nodes, their activation and degranulation around immobilized Mf may contribute to the microfilaricidal efficacy of the drug [5–7]. Trafficking of macrophages, eosinophils, memory and effector T and B lymphocytes is mediated by cytokines and chemokines [8], and following ivermectin treatment several chemotactic cytokines, notably those which regulate eosinophil activation such as IL-5, RANTES/CCL5 and eotaxin/CCL11, will raise shortly after treatment and these changes were correlated with systemic adverse reactions in patients [10,11]. Following ivermectin treatment, clinical reaction scores will peak at day 3 post-therapy [2], and here we sought to determine whether serum concentrations of CC chemokine will change and whether these alterations could be correlated with adverse responses. In the present study, MDC/CCL22 and TARC/CCL17 – these chemokines mediate preferentially memory Th2-type cell recruitment to dermal tissues [18] – increased transiently within days following ivermectin, while others – such as MIP-1α/CCL3 and MIP-1β/CCL4, which attract and activate Th1-type cells [8,19] – changed only after months post-initial treatment.

Sequestration and recruitment of monocytes and memory Th2-type cells to the skin, facilitated by MDC/CCL22, TARC/CCL17 and CTACK/CCL27 [18], appears to be a plausible mechanism by which anti-Mf responses may become initiated during the first days post-ivermectin. In contrast, the macrophage inflammatory chemokines MIP-1α/CCL3 and MIP-1β/CCL4 did not change during Mf clearance and augmented only after several months post-treatment. These chemokines promote macrophage-mediated Th1-type inflammatory responses [19] and, as they remained unchanged during the first days and the following months post-therapy, activation of macrophages by MIP-1α/CCL3 and MIP-1β/CCL4 may not have contributed essentially to the killing of microfilariae of *O. volvulus*.

Our observations suggest that next to eosinophils [10] several other leucocyte populations, i.e. macrophages as well as memory Th2-type lymphocytes and B cells, attracted and activated by MDC/CCL22 and TARC/CCL17, may contribute to dermal immune responses and Mf killing and Mf clearance. MDC/CCL22 and TARC/CCL17 are also considered as chemokines implicated in chronic inflammatory skin diseases [20], Th2-dominated inflammatory reactions in the skin [21]; they were found elevated with eosinophilic pneumonia [22] and in patients infested with *Paragonimus westermani* [23]. Anti-allergic drug treatment of atopic dermatitis patients decreased TARC/CCL17 and MDC/CCL22 levels [24] and systemic neutralization of TARC/CCL17 and MDC/CCL22 by specific antibody diminished pulmonary granuloma formation and its eosinophil content in sensitized mice challenged with live *Schistosoma mansoni* eggs [25]. In the patients from this study, MDC/CCL22 and TARC/CCL17 serum levels were clearly higher than in the infection-free European controls; indeed, as high as those found with atopic dermatitis and allergic asthma [26–28]. This supports that Th2 cells attracted to dermal tissues may contribute to the acute and chronic skin pathology as seen in onchocerciasis, i.e. papular rash, onchodermatitis, skin depigmentation and atrophy, but it remains uncertain whether these elevated chemokines predispose onchocerciasis patients for allergic disorders, e.g. atopy and asthma, especially as helminth infections were described to prevent the development of allergy [29–32]. Using logistic regression analysis we did not find a significant interaction of serum levels of MDC/CCL22 and TARC/CCL17 with clinical adverse reaction scores following ivermectin therapy; this may be important, because these chemokines may attract effector cells for parasite clearance without mediating clinical side effects. In contrast, the induction by ivermectin of chemokines such as RANTES/CCL5, IL-5, eotaxin/CCL11 – which activate eosinophils and promote their degranulation – correlated with the onset and severity of systemic adverse reactions [10,11].

In onchocerciasis patients, the levels of CTACK/CCL27 were strongly elevated compared to controls. CTACK/CCL27 is produced predominantly by epidermal keratinocytes; its function is to attract Th2-type memory and effector T and B lymphocytes into the epidermis from the peripheral blood and CTACK/CCL27 may help to determine the character and strength of local immune responses [18,27,33,34]. Elevated CTACK/CCL27 production and high serum levels were seen in atopic dermatitis [26] and CTACK/CCL27 was considered to be a skin-specific objective marker for clinical and laboratory parameters of atopic dermatitis [35]; however, treatment of atopic dermatitis patients with cyclosporin A did not change CTACK/CCL27 but increased TARC/CCL17 levels [27]. A similar kinetics for CTACK/CCL27 and TARC/CCL17 was seen in the ivermectin-treated *O. volvulus*-infected patients and therefore the contribution of CTACK/CCL27 and TARC/CCL17 to the severity of clinical manifestations in onchocerciasis should be investigated further. Following a single dose of ivermectin the continuously high production of CTACK/CCL27 and TARC/CCL17,

despite the reduction of Mf, could be due to the fact that the drug will not kill adult filariae of *O. volvulus*. The chemokines MDC/CCL22, TARC/CCL17 and CTACK/CCL27 mediate monocyte and lymphocyte recruitment to the skin and thus may contribute to anti-parasite cellular responses and the onchocerciasis-specific pattern of inflammatory skin disease. However, their transient changes and their lack of interaction with adverse effects following ivermectin supported that they may not promote hypersensitivity responses and chronic inflammatory skin diseases. Therefore, it is necessary to know how these immune mediators may change in onchocerciasis patients following repeated ivermectin therapy and in patients with other chronic helminth parasite infections, and also in allergy or atopic dermatitis patients during immunotherapy.

The CC chemokines TARC/CCL17 and MDC/CCL22 changed in patients shortly after ivermectin but returned to before-treatment levels. Before ivermectin, MIP-1α/CCL3 and MIP-1β/CCL4 levels – which attract and activate Th1-type cells [8] – were low and similar to healthy controls, but these CC chemokines changed after months of post-initial treatment and remained elevated. The association of MIP-1α/CCL3 and MIP-1β/CCL4 with a Type 1 immune response corresponds with their biological function to decrease Th2 cytokines, to reduce eosinophilia and airway hyperreactivity and to recruit macrophages into the sites of inflammation [8]. The levels of RANTES/CCL5, which has been allocated to Th1-type chemokines [19], remained widely unchanged in our study – only at 1 year post-therapy was RANTES/CCL5 found augmented in ivermectin-treated patients. In the present study, serum concentrations of RANTES/CCL5 in onchocerciasis patients were similarly high, as observed previously by Cooper and co-workers [10], but we did not find a transient increase of RANTES/CCL5 following ivermectin therapy. This was due probably to the transient expression and production of RANTES/CCL5, which returned to baseline levels within 72 h post-therapy [10]. The augmentation of MIP-1α/CCL3 and MIP-1β/CCL4 only after several months post-ivermectin may be caused by the slow re-invasion of *O. volvulus* Mf into the skin of patients, triggering delayed-type hypersensitivity reactions and a phagocyte-mediated host defence [19]. Proinflammatory effects have been described for both chemokines [36] and elevated levels of MIP-1α/CCL3 [37] were found associated with chronic schistosomiasis and the hepatosplenic form of the disease. Furthermore, in ivermectin-treated onchocerciasis patients, parasite antigen-specific cellular proliferation recovered gradually during the months following a single dose and the cytokine production by peripheral blood mononuclear cells (PBMC) changed towards a mixed Th1- and Th2-type profile [13]. This suggests that parasite densities and the state of infection, i.e. progressive *versus* regressive, may determine the extent by which chemokines mediate Th1 or Th2 type cell recruitment and activation.

In conclusion, following a single dose of ivermectin and during the rapid clearance of *O. volvulus* microfilariae, the serum levels of several chemokines, notably TARC/CCL17 and MDC/CCL22, changed in patients; those will attract and activate monocytes and Type 2 memory T lymphocytes and B cells, whereas other chemokines such as CTACK/CCL27 remained constitutively elevated or rose only several months post- treatment, as seen for MIP-1α/CCL3 and MIP-1β/CCL4. The transient changes of TARC/CCL17 and MDC/CCL22 were not associated with clinical adverse effects following ivermectin therapy, which supported that they may not promote hypersensitivity responses and chronic inflammatory skin diseases in onchocerciasis. The late rise of MIP-1α/CCL3 and MIP-1β/CCL4 following ivermectin indicated an adaptation of immune responses associated with persistent low levels of *O. volvulus* microfilariae and an expiring *O. volvulus* infection.

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